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Carbon disulfide effects on pre-baited vs. non-pre-baited rats exposed to low dosage zinc phosphide rodenticide bait

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Abstract

Control of rodents with rodenticides is frequently ineffective due to bait-shyness and neophobia. In an initial study, to increase bait acceptance, Wistar-strain laboratory rats were individually tested for attraction to 3 odors (rat urine, preputial gland extract, and carbon disulfide) and for repellence to a fourth odor (coyote urine) in a 2-compartment arena. Three measures of rat preference-amount of bait consumed, elapsed time to feeding, and time in each compartment suggested that carbon disulfide at 10 ppm was an attractant ($p = 0.003$). The other odors produced inconsistent results. In a second study, rats were offered pre-weighed quantities of USA Environmental Protection Agency (EPA) standard challenge bait containing a low level of 0.20% zinc phosphide (ZP) rodenticide. Two groups of rats ($n = 28$) were given either 0 or 3 days of pre-baiting prior to ZP bait exposure. Half the animals in each group received deionized water placebo odor and the others received the carbon disulfide odor in the presence of baits. EPA bait with carbon disulfide odor produced elevated consumption ($p = 0.0099$) with females showing a greater degree of effect ($p = 0.046$). Pre-baiting produced higher mortality (220%) and higher level ZP dosages (30%) during baiting. Although mortality was 11% higher in rats presented ZP bait with carbon disulfide, dosages were unchanged. Relative to pre-baiting, the attractant had a marginal effect on ZP bait acceptance and produced mortality changes in males only. Further development of controlled release of this attractant for rodenticide baits would be needed before field applications are attempted; implications for using attractants in baits, traps, tracking powders to optimally control both rat genders are discussed. Published by Elsevier Science Ltd.

Keywords: Attractant; Baits; Bait-shyness; Carbon disulfide; Odor; Rodenticide

1. Introduction

Rodent control with baits and traps in crop lands is often ineffective in reducing rodent densities due to several factors. Poor bait acceptance, sub-lethal dosing, social feeding, and dietary preferences can reduce the efficacy of rodenticide baiting (Berdoy and MacDonald, 1991). Frequently, a lack of attraction to the baiting or trapping sites is due to poor placement or inadequate pre-baiting.

Although zinc phosphide (ZP) has been reported to be an effective acute rodenticide (Hood, 1972; Matschke et al., 1982; Sterner et al., 1996), numerous researchers have also reported bait acceptance problems related to bitter taste, sub-lethal toxicosis, and subsequent conditioned aversion after rodents have ingested minimal levels of ZP bait (Sridhara, 1983; Prakash and Ghosh, 1992; Reidinger, 1995). Bait-shyness, induced through conditioned taste aversion, can last more than a year, even when the ZP has been removed from the baits (Shepard and Inglis, 1993). In addition, the bitter taste of ZP sometimes makes it effective only when there is little or no alternative food available other than the rodenticide baits. In no-choice tests, ZP often produces 100% mortality, but in 2-choice tests that more closely simulate its use in crop protection, mortality levels can range from 50% to 75% (i.e., frequently below the 70% efficacy level suggested for USA-EPA rodenticide registration). Several researchers have also noted the need for an attractant odor that could be added to ZP baits to successfully compete with the alternate field crop foods (Koehler et al., 1994; Reidinger, 1995). Attractive baits for problem rodents could also improve the selectivity for target rodent species by reducing the bait exposure periods (Watkins et al., 1999).

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Despite several published reports indicating many sources of putative attractive or repellent odors (Marsh, 1988; Koehler et al., 1994; Mason et al., 1994; Mason, 1997; Witmer et al., 1997), only a few laboratory and field studies have empirically evaluated their effects on improving either rodenticide baiting or trapping effectiveness (Burwash et al., 1998a, b). The present study was designed to assess the improved baiting efficacy using ZP rodenticide in conjunction with a selected attractant (e.g. carbon disulfide odor, preputial gland extract, or rat urine) and/or a repellent, predator urine odor, to drive the rodents away from crops and toward baits in laboratory tests with albino rats. Field studies with wild rats would be needed to further confirm the present findings and to refine or optimize the use of odor agents for improved rodent damage reduction in field crops.

2. Materials and methods

2.1. Animals

Adult Wistar-strain albino rats (30 males—body mass range = 138.7–155.8 g; 30 females—body mass range = 145.0–173.8 g) were purchased from Simonsen Laboratories, Inc. (Gilroy, CA). Rats were housed in individual suspended cages with rodent food and water available ad libitum throughout the studies. Animals were maintained on a 12 h reversed light cycle with the overhead fluorescent lights off at 0600 and on at 1800 MST. Room temperatures were controlled to range between 23°C and 27°C with relative humidity levels uncontrolled but low (i.e., typically <30%).

2.2. Arena tests

To select a candidate attractant or repellent that would consistently alter rat feeding behavior, two arenas (150 cm × 60 cm × 75 cm) for observing individual animals were constructed with clear plexiglass panels. In the center of each arena there was a double-walled partition that contained openings allowing animal access to both sides of the arena. Spill-proof food cups, each containing 40 g of USA-EPA challenge bait (standard mixture of 5.0% corn oil, 5.0% powdered sugar, 65% ground corn, and 25% ground rolled oats), were attached with spring clips to the far walls of each arena. Animals were individually observed by videotape for the 60 min sessions with de-ionized water (DI) presented as a placebo odor material in a 15 ml glass vial fastened with adhesive tape to a bait-station food cup on a randomly chosen arena side. Simultaneously, a test odor (preputial gland extract, rat urine, coyote urine, or carbon disulfide) was presented in a 15 ml glass vial fastened with adhesive tape to the second bait station food cup on the opposite side of the arena. The carbon disulfide solution was presented successively over 4 levels (0.1, 1.0, 10.0 and 20.0 ppm) to evaluate relative odor attraction in different groups of rats ($n = 8$). An electronic balance was used to weigh bait cups so that mass of consumed bait could be calculated and used as the main indicator of attractant or repellent odor effects. Digital-video tape recordings were also examined to measure time events (e.g., seconds until initial rat contact of each bait container, total time in each arena side, average feeding bout time).

2.3. Rodenticide baiting tests

To determine whether a selected attractant as detected in the arena test would also alter rodenticide baiting efficacy, animals were randomly selected (7 males; 7 females) for each of 4, 14-animal groups. Two groups received either an odor attractant (10 ppm carbon disulfide in DI water) or the placebo odor (DI) placed adjacent to the EPA bait material during both a 3-day pre-bait phase (7 h per day) and on the ZP bait day (24 h). Odor materials were presented under the food cup covers using filter paper wicks saturated with 2 ml of each agent (see Fig. 1), so that the odors would be dispersed surrounding the top and inside of the cups as the animals fed. Two remaining groups received no pre-baiting, but were presented with either the carbon disulfide or DI water odors when first presented on the ZP bait day. A ZP concentration of 0.2% (mass/mass) in the EPA baits rather than the standard 2.0% (mass/mass) level was chosen to ensure that only around one-half of the rats in the DI water group would receive a lethal dose over the 24 h exposure, thus allowing a more sensitive efficacy assessment of the attractant odor effect. For Norway rats, the LD_{50} value of 27.0 mg/kg (Hood, 1972) was used to provide an index of prediction for mortalities in our study. An alternate food cup containing untreated EPA bait was also available to each rat for all bait exposures. Bait consumption levels for each animal were measured and used with individual body weights to calculate the individual mg/kg dosages of ZP ingested. Animals that survived ZP baiting were euthanized within 4 h after exposure to minimize rodenticide-induced pain or stress.

2.4. Data analyses

For the screening trials, attractant or repellent effects were analyzed by comparing animal intake of EPA bait placed on each arena side during the 60 min sessions in the arenas. A one-way analysis of variance (ANOVA) was performed to detect preference or repellent effects generated by each odor. In cases where significant effects were observed, video tapes of the sessions were analyzed to measure the seconds of elapsed time until the animals initially fed in each side of the arena, the

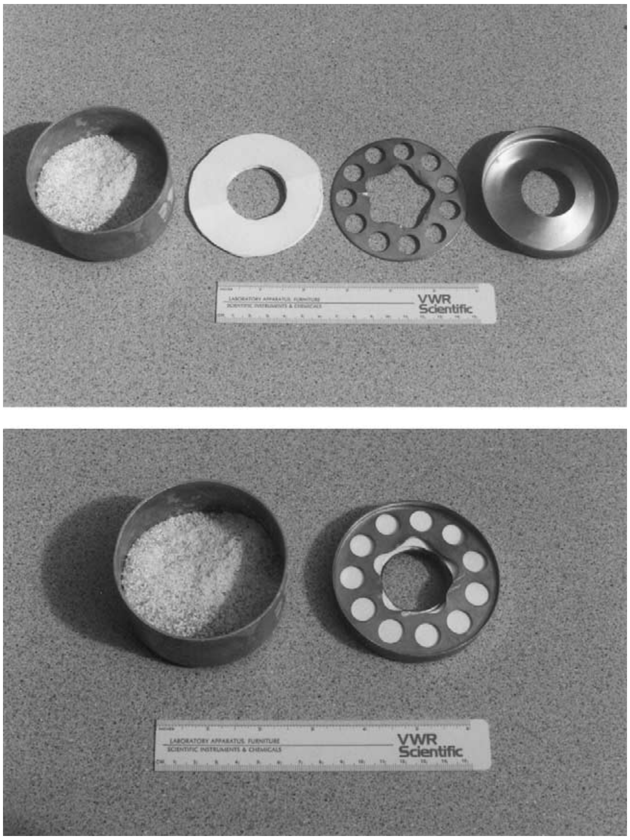

Fig. 1. Upper photograph. From left to right: metal food cup containing EPA challenge bait, round filter paper with centered opening, spillage screen with center opened, food cup cover. Lower photograph: configuration ready to be placed on top of metal food cup as an odor dispersion device to be used with 2 ml of 10 ppm carbon disulfide or DI water introduced via 3 ml syringe through the spillage screen openings.

total time spent feeding at each food cup, and the total times spent in each arena side. For the rodenticide baiting tests, bait consumption levels in the presence of the selected odor attractant (10 ppm carbon disulfide) and placebo (DI water) odor were tabulated on a daily basis. Treatment × day ANOVAs were performed for the baiting day with mg/kg ZP intakes calculated for each animal based on 0.2% active ingredient in EPA bait. Gender × treatment group ANOVAs were performed for rodenticide dosages and bait consumption.

3. Results

3.1. Arena tests

One of the tested odor materials, carbon disulfide at 10 ppm, consistently produced more EPA bait consumption compared to untreated EPA bait ($F = 18.34$, df = 1,14, $p = 0.003$). Other odor materials and other levels of carbon disulfide produced no statistically detected effects on feeding or rat movements in the arenas (Table 1). Based on means, animals in the 10 ppm group also showed a more rapid response time (i.e., less elapsed time: −29%) until their first feeding and more time expended (+29%) in exploring the odor-treated side of the arena. However, coefficients of variation were relatively high ($CVs = 0.52$ and 0.73, respectively) and these effects were not statistically significant ($p > 0.25$, both measures). The replication of the 10 ppm carbon disulfide odor test again indicated a preference effect on the consumption measure ($F = 13.43$, df = 1,10, $p = 0.007$). In addition, females consumed more EPA bait in the presence of carbon disulfide than did males ($F = 7.56$, df = 1,10, $p = 0.046$).

Table 1
Mean ± SE consumption levels of EPA bait offered for 60 min in the observation test arenas

Test substance	Odor-treated bait (g)	DI water odor bait (g)
Rat urine	1.84 ± 0.26	1.34 ± 0.31
Coyote urine	1.27 ± 0.13	1.85 ± 0.36
Preputial gland	1.38 ± 0.49	1.73 ± 0.20
Carbon disulfide (ppm)		
0.1	1.51 ± 0.31	2.19 ± 0.45
1.0	2.29 ± 0.36	2.06 ± 0.42
10.0[a]	3.42 ± 0.51	1.28 ± 0.21
20.0	1.02 ± 0.35	1.15 ± 0.38

[a] $p = 0.003$.

3.2. Pre-bait consumption

Table 2 gives the daily pre-bait consumption levels for EPA bait with either 2 ml of 10 ppm carbon disulfide odor or DI presented under the food cup covers on filter paper wicks. Both odor treatment effects ($F = 7.75$, df = 1,52, $p = 0.0099$) and a day effect ($F = 10.25$, df = 2,52, $p = 0.0002$) were detected. Consumption levels of carbon disulfide treated bait were higher on days 2 and 3 compared to day 1 suggesting an initial neophobic response. Both males ($F = 4.90$, df = 2,24, $p = 0.0164$) and females ($F = 6.17$, df = 2,24, $p = 0.0069$) showed carbon disulfide bait consumption increases over the 3-day pre-bait period; females also showed significant ($F = 5.39$, df = 2,24, $p = 0.0117$) DI bait consumption increases over the 3 pre-baiting days. Both genders showed total (treated + alternate) bait intake (g/kg) increases in the CS group over the DI group, but again this increase was only significant ($F = 8.58$, df = 1,12, $p = 0.0126$) for females.

3.3. ZP bait consumption

Table 3 gives the mean level of ZP mg/kg consumed and mortality percentages after the 24 h bait exposure

Table 2
Mean ± SE treated bait consumption levels comparing placebo (DI) with 10 ppm carbon disulfide odors for each pre-baiting day. Total treated and alternate bait consumption levels are also shown for the 3-day period. Values are in grams of bait consumed and grams of bait consumed per kilogram of body weight (g/kg)

Bait type	Day	Males	Females	Combined
Daily treated bait consumption (grams and (g/kg))				
CS[a]	1	7.14 ± 1.42* (15.57 ± 2.85)	6.02 ± 0.68* (20.81 ± 2.51)	6.71 ± 0.81* (18.19 ± 2.03)
	2	11.99 ± 1.26 (26.04 ± 2.86)	9.15 ± 1.30 (31.60 ± 4.81)	10.57 ± 0.98 (28.82 ± 2.91)
	3	11.11 ± 1.06 (24.26 ± 2.56)	10.17 ± 1.00 (34.62 ± 3.18)	10.64 ± 0.74 (29.44 ± 2.47)
DI[b]	1	5.13 ± 0.92 (11.53 ± 2.14)	4.76 ± 1.00* (18.26 ± 3.70)	4.95 ± 0.96* (14.89 ± 2.32)
	2	9.04 ± 1.92 (20.44 ± 4.30)	6.46 ± 0.69 (24.78 ± 2.71)	7.75 ± 1.52 (22.61 ± 2.61)
	3	7.84 ± 1.77 (13.67 ± 2.77)	7.47 ± 1.25 (28.94 ± 5.18)	7.66 ± 1.53 (21.31 ± 3.58)
Total three-day consumption of treated plus alternate bait (grams and (g/kg))				
Bait type		Males	Females	Combined
CS[c]		20.13 ± 0.52 (42.83 ± 1.09)	17.28 ± 0.66* (59.28 ± 2.96)	18.71 ± 0.56* (51.06 ± 2.71)
DI		17.56 ± 1.26 (39.58 ± 3.17)	11.97 ± 0.83 (45.84 ± 3.04)	14.76 ± 1.06 (42.71 ± 2.35)

[a] CS bait consumption increased over the 3 pre-bait days for both genders.
[b] DI placebo bait consumption increased over the 3 pre-bait days, but only significantly for females.
[c] CS increased total three-day consumption overall, but only significantly for females.
*$p < 0.02$.

Table 3
Mean ± SE mg/kg zinc phosphide rodenticide dosages consumed by each of 8 rat groups and subsequent percentages of mortality within 24 h of bait exposure

	Pre-baited	Non-pre-baited
Carbon disulfide		
Males	19.63 ± 4.37 (57.1%)	9.92 ± 3.24 (0.00%)
Females	36.05 ± 9.60 (71.4%)	19.13 ± 2.88 (28.6%)
Deionized water		
Males	15.99 ± 3.21 (14.3%)	20.75 ± 3.65 (0.00%)
Females	37.14 ± 5.02 (85.7%)	20.37 ± 3.29 (42.9%)

for each of the 8 groups. Pre-baited females increased their ZP bait consumption 84% compared to non-pre-baited females, resulting in a 2-fold increase in mortality (5:14 vs. 11:14). The males showed no increase in ZP consumption when pre-baited (5:14 vs. 5:14), but their mortality increased from 0.0% to 57.1% (0:7 vs. 4:7) when pre-baiting was combined with the carbon disulfide odor attractant and from 0.0% to 14.3% (0:7 vs. 1:7) when pre-baiting was combined with the placebo odor.

Analysis of the mg/kg dose level consumption by each gender under each treatment condition (i.e., pre-baited vs. non-pre-baited groups and carbon disulfide and placebo sub-groups) detected a gender effect ($F = 11.09$, df = 1,22, $p = 0.003$) and an indication of a treatment × gender interaction (not significant ($F = 2.97$, df = 3,22, $p = 0.0551$); however, this was definitely worthy of closer examination (Tacha et al., 1982) and possibly indicated the males had increased ZP dosages toward lethal levels when the attractant odor, in contrast to DI, was presented during pre-baiting and baiting. In comparison, females were completely unaffected by the attractant odor, and were, instead, most affected by the pre-baiting procedure. That is, non-pre-baited mean ZP dosages were 19.13 and 20.37 mg/kg vs. 36.05 and 37.14 mg/kg for pre-baited females give either carbon disulfide or DI odor associated with ZP bait, respectively.

4. Discussion

Our observational and quantitative data from the arena study supported findings of previous studies (Galef et al., 1988; Mason et al., 1988; Mason, 1997) indicating increased Norway rat food detection, acceptance, and consumption with carbon disulfide present at 10 ppm. This compound was also found as a component odor in the breath of rats and mice (Galef et al., 1988; Mason et al., 1994). Theoretically, it acts as a safety signal odor when rodents encounter new foods or foods in new places, thereby reducing their neophobic reactions and enhancing their food sampling, acceptance, and consumption levels. Food odors made familiar to rodents can also reduce bait container neophobia in wild rats (Watkins et al., 1999). Our findings were also consistent with those of Mason et al. (1994) and Bean et al. (1998) in terms of carbon disulfide odor producing more attraction in female than in male Norway rats.

Our ZP baiting study, however, yielded mixed results. Carbon disulfide odor at 10 ppm produced an increase in male rat mortalities during ZP bait exposure, but only when accompanied with the 3-day pre-baiting procedure. When pre-baiting was accompanied with carbon disulfide, males increased their dosages of ZP to a minor degree when compared to DI animals (19.63 vs.

15.99 mg/kg, respectively). Female rats showed no increase in mortality in the presence of carbon disulfide odor regardless of whether or not they had been pre-baited; dosages of ZP remained unchanged for these animals (36.05 vs. 37.14 mg/kg for carbon disulfide vs. DI, respectively). During pre-baiting, however, females in our second study showed a higher gain in overall 3-day bait consumption in the presence of carbon disulfide compared with the alternate non-odor treated bait (59.28 vs. 45.84 g/kg) than did the males (42.83 vs. 39.58 g/kg), again indicating the gender difference shown in the arena study. This finding implied that higher pre-baiting intakes and bait preferences are not predictive of higher ZP baiting intakes and subsequent mortality percentages when dealing with bait odor attractants. Overall, pre-baiting in our second study increased consumption of rodenticide bait 82–88% in female rats but not in males. The carbon disulfide odor produced a 22% increase in ZP dosage in pre-baited males, but it was also associated with a 52% decline in ZP dosage in the non-pre-baited males. In terms of dosages of ZP, carbon disulfide odor had only minor, inconsistent effects regardless of whether animals had been pre-baited or non-pre-baited. Thus, respective mortality changes for the pre-baiting condition (220% increase) and carbon disulfide odor presence (11% increase) indicated that this attractant produced only marginal improvement in ZP baiting efficacy.

Although the proportion of exposed rats succumbing to ZP (i.e., mortality ratio) increased to a minor degree in the carbon disulfide (12:28) vs. the placebo (9:28) odor groups when each was exposed to the 0.2% ZP baits, differences in their ingested mg/kg dosages were not detected statistically. This could have been an indication that the rats increased their initial rates of ingestion of the rodenticide bait in the presence of the attractant. A more rapid consumption rate for ZP or anticoagulant rodenticide baits may lead to a lessened need to leave baits exposed to non-target species over extended time periods (Watkins et al., 1999). Questions still remain, however, regarding efficacy effects of carbon disulfide odor when used with 2.0% ZP which is the level registered for field rodent control applications (Sterner et al., 1996). Effects of using odor attractant baits also need to be evaluated in non-target animals in future studies. In addition, studies are needed to confirm and examine the degree of the attractant effects in wild rodent species in agricultural situations when a variety of alternate food sources and attractive odors could compete with rodenticide baits. Because a relatively narrow range of carbon disulfide concentration was found to produce attraction in Norway rats, odor dispersing systems or controlled release methods would be needed for proper field dispersal of the odor. At this point in development, the field practicality of the carbon disulfide material as a means of improving ZP baiting efficacy remains unevaluated.

Bait formulation problems could, however, be predicted to present difficulties in maintaining the attractant effects. For example, when 10 ppm carbon disulfide in water was added directly to cracked corn bait using a starch xanthate carrier Koehler et al. (1994) were not able to demonstrate increased bait consumption by 3 wild rat species. The authors indicated that either the odor had been dissipated rapidly over the course of their experiments, or the bait formulation may have masked the carbon disulfide attractant odor. It is also possible that odor quality and bait taste mixture components could have been altered during their formulation procedure.

In terms of management implications, pre-baited male rats in our study were shown to be most affected by carbon disulfide odor when the ZP baits were presented and this was reflected in their mg/kg dosages and in their increased mortality ratios. Pre-baiting had a strong effect on females, but the presence of carbon disulfide odor did not lead to any additional increase in their mortality ratios. Females did, however, show a stronger, initial attraction to carbon disulfide odor in the short term 60 min arena tests and during pre-baiting in the ZP test. Our observations therefore lead to the prediction that the use of ZP baits with pre-baiting and carbon disulfide odor will be more effective in reducing the number of males. Females are predicted to be affected only by the pre-baiting procedure regardless whether carbon disulfide is present or absent. On the other hand, carbon disulfide could be used to an advantage with traps and tracking powers to attract proportionately more females. The pre-baiting and baiting effects would probably be amplified in situations that generate neophobic responses (e.g., the appearance of new bait flavors amongst a stable crop as a food source). In some situations, it may be possible to integrate these gender-specific attractive properties of carbon disulfide with other rodenticides, traps, or tracking powders to optimize local population control of both genders.

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